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Use of inhibitors of enzymes having activities of amino peptidase N and/or dipeptidyl peptidase IV and of pharmaceutical preparations thereof for a therapy and prevention of dermatological diseases with sebaceous hyperproliferation and modified conditions of differentiation

The invention describes the inhibition of the DNA synthesis necessary for the proliferation of sebaceous cells (sebocytes) by the action of inhibitors of amino peptidase N (APN; E.C. 3.4.11.2.; CD13) and/or of dipeptidyl peptidase IV (DP IV; E.C. 3.4.14.5.; CD26) as the result of the separate, of the simultaneous or, with respect to the time, of the immediately successive application of respective specific inhibitors of these enzymes or of inhibitors of enzymes having a similar substrate specificity (APN- and/or DP IV-analogous enzyme activity) on the basis of amino acid derivatives, peptides or peptide derivatives by which the proliferation (DNA synthesis) of sebocytes is suppressed.

A number of dermatological diseases are associated with hyperproliferation and modified states of differentiation of sebocytes. Among them are both benign follicular hyperproliferation conditions (acne, acneiform follicular reactions, steatocystoma multiplex, naevi of sebaceous glands, senile sebaceous gland hypertrophy, seborrhea of the skin and of the hair) and malign follicular hyperproliferation conditions (mixed tumors, sebaceomes, sebaceous gland tumors, sebaceous gland CA).

Peptidases, as for example, dipeptidyl peptidase IV and amino peptidase N or similarly acting enzymes are of particular interest for a regulation and/or modulation of interactions between cells, since they are, in part, localized in the plasma membrane of the cells as ectoenzymes, interact with other extracellular structures, activate or inactivate peptiderge messenger substances by enzyme-catalyzed hydrolysis, and, hence, are important for a cell-to-cell communication. [Yaron A., et al.: Proline-dependent structural and biological properties of peptides and proteins. Crit. Rev. Biochem. Mol. Biol. 1993; 28 : 31 - 81;

Vanhoof G., et al.: Proline motifs in peptides and their biological processing. FASEB J. 1995; 9: 736 - 744].

It was shown that membrane-allocated peptidases like DP IV or APN play a key role in the process of an activation and clonal expansion of immune cells, in particular of T-lymphocytes. [Fleischer B.: CD26 a surface protease involved in T-cell activation. Immunology Today 1994; 15: 180 - 184; Lendeckel U. et al.: Role of alanyl aminopeptidase in growth and function of human T cells. International Journal of Molecular Medicine 1999; 4: 17 - 27; Riemann D. et al.: CD13 - not just a marker in leukemia typing. Immunology Today 1999; 20: 83 - 88]. Several functions of mitogene-stimulated mononuclear cells (MNZ) or of enriched T lymphocytes as, for example DNA-synthesis, production and secretion of immunostimulating cytokines (IL-2, IL-6, IL-12, IFN-γ) and helper functions for B-cells (IgG synthesis and IgM synthesis) may be inhibited in the presence of specific inhibitors of DP IV or of APN [Schön E., et al.: The dipeptidyl peptidase IV, a membrane enzyme involved in the proliferation of T lymphocytes. Biomed. Biochim. Acta 1985; 2: K9-K15; Schön E., et al.: The role of dipeptidyl peptidase IV in human T lymphocyte activation. Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and immunoglobulin synthesis in vitro. Eur. J. Immunol. 1987; 17: 1821-1826; Reinhold D., et al.: Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor β1 in PWM-stimulated PBMNC and T cells. Immunology 1997; 91: 354-360; Lendeckel U., et al.: Induction of the membrane alanyl aminopeptidase gene and surface expression in human T-cells by mitogenic activation. Biochem. J. 1996; 319: 817-823; Kähne T., et al.: Dipeptidyl peptidase IV: A cell surface peptidase involved in regulating T cell growth (Review). Int. J. Mol. Med. 1999; 4: 3-15; Lendeckel U., et al.: Role of alanyl aminopeptidase in growth and function of human T cells (Review). Int. J. Mol. Med. 1999; 4: 17-27].

It is already known that a treatment of autoimmune diseases and transplant rejection may be achieved by an inhibition of dipeptidyl peptidase IV localized on immune cells by means of synthetic inhibitors (see, for example, EP-A 0 764 151; WO 095/29,691; EP-A 0 731 789; EP-A 0 528 858).

The invention is based on the surprising finding that the single or simultaneous effect of inhibitors of the dipeptidyl peptidase IV/CD26 and/or inhibitors of the amino peptidase N/CD13 or of inhibitors of enzymes having a similar substrate specifity (APN- and/or DP IV-analogous enzyme activity), expressed on or in sebaceous cells (sebocytes) inhibits the proliferation (DNS synthesis) of these cells.

Our invention shows that, for a therapy and for a prevention of dermatological diseases with sebaceous hyperproliferation and modified conditions of differentiation (benign follicular hyperproliferation conditions like acne, acneiform follicular reactions, steatocystoma multiplex, naevi of sebaceous glands, senile sebaceous gland hypertrophy, seborrhea of the skin and of the hair as well as malign follicular hyperproliferation conditions like mixed tumors, sebaceomes, sebaceous gland tumors, sebaceous gland CA) for the generation of which the proliferation of sebocytes has a central importance, the single or simultaneous application of inhibitors of DP IV and of APN or of inhibitors of enzymes having a similar substrate specificity (APN- and/or DP IV-analogous enzyme activity) or of corresponding pharmaceutical preparations and dosage forms thereof is suitable.

In detail, the invention is based on the findings that the DNA synthesis of sebaceous cells (sebocytes) is significantly inhibited by the administration of inhibitors of dipeptidyl petidase IV and/or of inhibitors of amino peptidase N.

Up to now, the above mentioned diseases are treated topically and/or systemically by administering antibiotics and/or antiproliferative and differentiating substances (antiandrogens, 13-cis-retinic acid and others). In the systemical treatment in particular, undesired side effects are often observed, inter alia teratogenicity, lipid metabolic disorders, psychoreactive phenomena, gastrointestinal disorders as well as muco-cutane irritative reactions.

The use of DP IV and/or APN inhibitors would represent a completely new, presumably very effective, possibly cost effective therapy form and a valuable alternative component of existing therapy concepts of the above-referenced diseases.

The inhibitors of dipetidyl peptidase IV and/or the inhibitors of amino peptidase N or inhibitors of enzymes having a similar substrate specificity (APN-analogous and/or DP IV-analogous enzyme activity) applied according to the invention may be administered in pharmaceutically applicable formulation complexes as inhibitors, substrates, pseudo substrates, inhibitory active peptides and peptide derivatives as well as antibodies to those enzymes.

Preferred effectors for DP IV, are for example, Xaa-Pro-dipeptides, corresponding derivatives, preferably dipeptide phosphonic acid diaryl esters and their salts, Xaa-Xaa-(Trp)-Pro-(Xaa)n peptides (n = 0 to 10), corresponding derivatives and their salts or amino acid (Xaa)-amides, corresponding derivatives and their salts, wherein Xaa is an α-amino acid/imino acid or an α-amino acid derivative/imino acid derivative, preferably N^{ε}-4-nitrobenzyl oxycarbonyl-L-lysine, L-proline, L-tryptophane, L-isoleucine, L-valine, and cyclic amines, e. g. pyrrolidine, piperidine, thiazolidine, and their derivatives act as amide structure. Such compounds and their preparation were described in an earlier patent (K. Neubert et al. DD 296075A5). Preferred inhibitors for the alanyl amino peptidase are bestatin (ubenimex), actinonin, probestin, phebestin, RB3014 or leuhistin.

The inhibitors are administered simultaneously with known carrier substances. On the one hand, the administration occurs as a topical application in the form of, for example, creams, ointments, pastes, gels, solutions, sprays, liposomes and nanosomes, lotions (agitated mixtures), hydrocolloid dressings, plasters and similar novel carrier substrates, jet injections or other dermatological bases/vehicles, including instillative applications, and on the other hand, as a systemic application for oral, transdermal, intravenous, subcutaneous, intracutaneous, intramuscular use in suitable formulations or in a suitable galenic form.

Example 1

Inhibition of the DNA synthesis of the immortalized human sebaceous cell line SZ95 by the incubation with synthetic inhibitors of DP IV and/or APN

Our investigations show that the DNA synthesis of the immortalized human sebaceous cell line SZ95 (Zouboulis, C.C. et al.: Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95), J. Invest. Dermatol. 1999, 113, 1011 – 1020) is inhibited by the administration of inhibitors of the DP IV (Lys[Z(NO_2)]-thiazolidide and/or of the APN (actinonin) in a dose-dependent manner.

The human sebaceous cell line SZ95, which is accepted as a cell model for acne, expresses strongly DP IV and APN (Figure 1). The enzyme activity of the DP IV of vital cells amounts to 38 ± 18 pkat/10^6 cells, and that of the APN amounts to 262 ± 58 pkat/10^6 cells (n = 3). Accordingly, the mRNA of APN and DP IV is detectable on these cells (Figure 2).

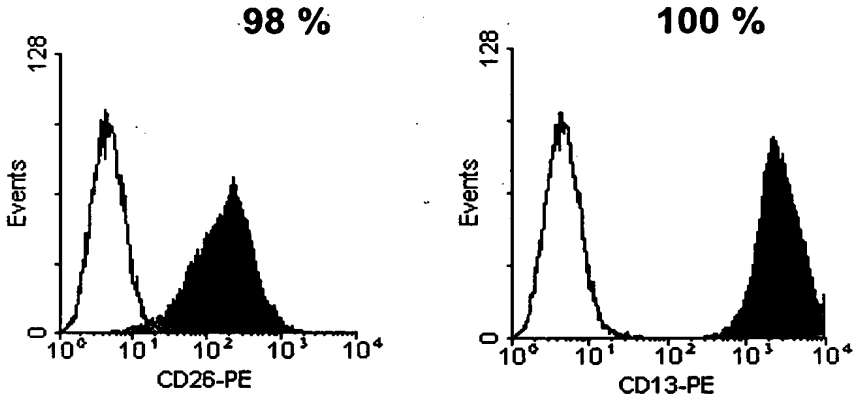

Figure 1: Cytometric flow rate verification of the expression of DP IV (CD26) and APN (CD13) on SZ95 cells

SZ95 cells were 48 h incubated with the above-mentioned inhibitors and subsequently the DNA synthesis was determined by the measurement of the ^{3}[H]-Thymidine incorporation

as described in Reinhold et al. (Reinhold, D. et al.: Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor ß1 in PWM-stimulated PBMNC and T-cells; Immunology, 1997, 91; 354 – 360). Figure 2 shows the inhibition of the DNA synthesis depending on the dose.

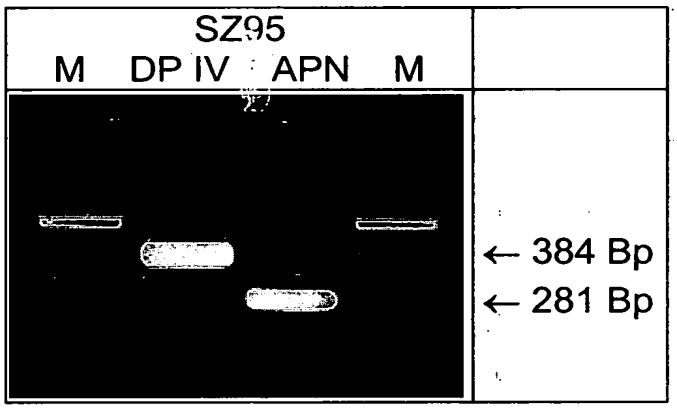

Figure 2: Verification of the mRNA expression of DP IV (CD26) and APN (CD13) on SZ95 cells via RT-PCR

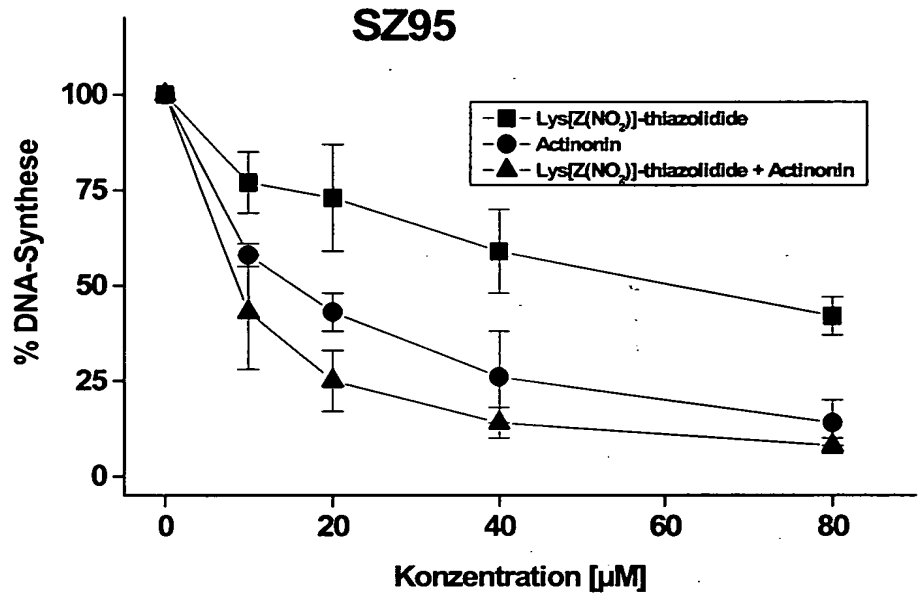

Figure 3: Effect of inhibitors of the DP IV (Lys[Z(NO_2)]-thiazolidide) and of the amino peptidase N (actinonin) on the DNA synthesis of human SZ95 sebaceous cells depending on the dose.

The cells were 48 h incubated with inhibitors in the above-mentioned concentrations. Subsequently ^{3}[H]-Methyl-thymidin was added to the culture medium. After 6 further hours the amount of ^{3}[H]-Thymidine incorporated in the DNA was measured.